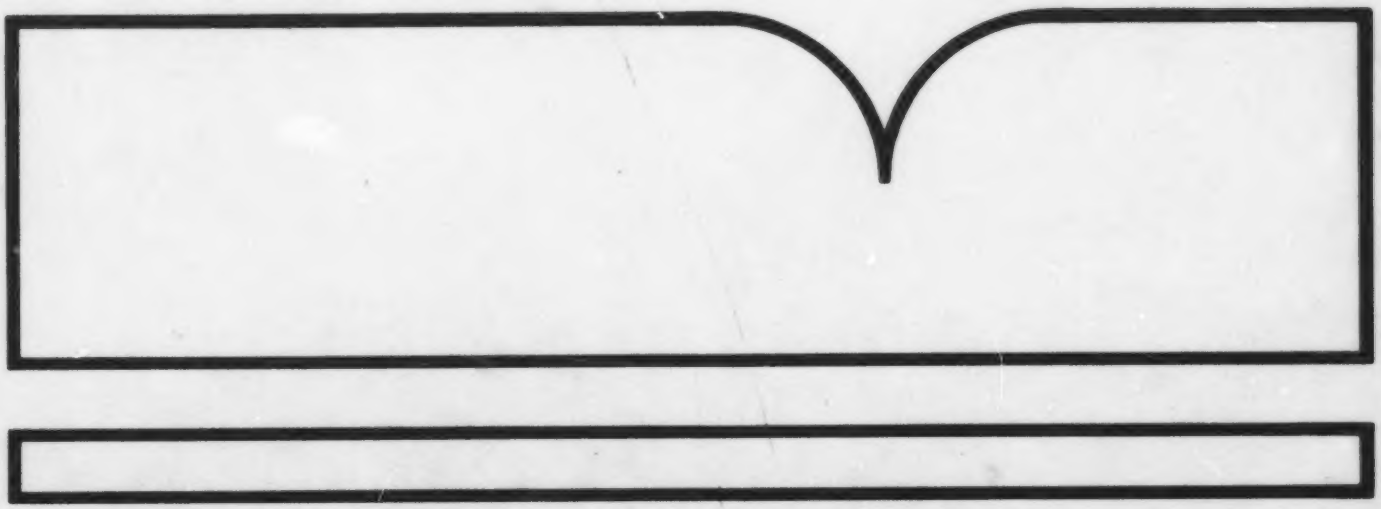
PB81-165326

Chironomidae Toxicity Tests--Biological Background and Procedures

(U.S.) Environmental Research Lab.
Duluth, MN

1980



U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completin

1. REPORT NO.	2.	3. PB81-165376
EPA-600/J-80-217	Journal Article	

4. TITLE AND SUBTITLE	5. REPORT DATE
Chironomidae Toxicity Tests -- Biological Background and Procedures	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
R. L. Anderson	
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, Minnesota 55804	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES
IN: Aquatic Invertebrate Bioassays, ASTM STP 715; A. L. Buikema, Jr., and John Cairns, Jr. (eds.); American Society for Testing and Materials, pp. 70-80 (1980).

16. ABSTRACT

Toxicity tests must be based on an understanding of the test animal's life cycle. The first section of this report describes the biological information needed to develop toxicity test procedures. The second section describes three categories of toxicity test systems - short-exposure, partial life-cycle, and life-cycle exposures.

Each researcher has developed a procedure to solve a particular problem. Therefore, it is impossible to provide specific recommendations for a general toxicity test. In practice, anyone using chironomids should establish a clear experimental objective and then select the test species. The required biological information and test procedure can then be obtained from the literature or developed by experimentation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	11
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA-600/J-80-217
Journal Article

Authorized Reprint from
Special Technical Publication 715

American Society for Testing and Materials
1916 Race Street, Philadelphia, Pa. 19103
1980

R. L. Anderson[1]

Chironomidae Toxicity Tests—Biological Background and Procedures

REFERENCE: Anderson, R. L., **"Chironomidae Toxicity Tests—Biological Background and Procedures,"** *Aquatic Invertebrate Bioassays, ASTM STP 715.* A. L. Buikema, Jr., and John Cairns, Jr., Eds., American Society for Testing and Materials, 1980, 70–80.

ABSTRACT: Toxicity tests must be based on an understanding of the test animal's life cycle. The first section of this report describes the biological information needed to develop toxicity test procedures. The second section describes three categories of toxicity test systems—short-exposure, partial life-cycle, and life-cycle exposures.

Each researcher has developed a procedure to solve a particular problem. Therefore, it is impossible to provide specific recommendations for a general toxicity test. In practice, anyone using chironomids should establish a clear experimental objective and then select the test species. The required biological information and test procedure can then be obtained from the literature or developed by experimentation.

KEY WORDS: chironomidae, toxicity tests, aquatic invertebrate bioassays, life cycle, chironomid biology, invertebrates, bioassays, aquatic toxicology

Chironomids are an ecologically diverse family of Dipterans and are probably the most ubiquitous of all aquatic insects. They are found in the Arctic and Antarctic, in cold or warm water, in fresh water and on the edges of the oceans, in mud, on the surface of mud, and in cases or free living. Over 5000 species have been described, and large areas of the world have not been examined [*1*].[2] Because of this ecological and taxonomic diversity, it is necessary to limit the scope of any report on these insects. This report is an attempt to describe a minimum data base for toxicity testing and to catalog and describe some of the published toxicity test procedures or other test systems that could be adapted for toxicity testing.

Test procedure development has occurred in response to two needs. The

[1] Research entomologist, U.S. Environmental Protection Agency, Environmental Research Laboratory—Duluth, Duluth, Minn. 55804.

[2] The italic numbers in brackets refer to the list of references appended to this paper.

first need had economic origins with the goal of producing efficient control agents and dosing procedures to suppress nuisance mass emergences. In general, these tests have been acute (4 days or less) exposures using fourth instar larvae in a static system. The second need has developed because of an increase in the awareness of the importance of chironomids in freshwater ecosystems. This awareness, coupled with certain chironomid life-cycle characteristics, such as easily identified stages, short life-cycle time, and the ability to be reared in the laboratory, has resulted in an increase in the value of these insects for toxicity testing. The test systems that were developed to determine the effects of toxic compounds other than pesticides on these insects were usually longer than 4 days and included life-cycle events such as hatching, molting, or emergence.

A prerequisite for either approach is an understanding of the biological requirements of the test species. A central question for any testing procedure is: What kind and how much biological information is required before a test protocol can be developed? A minimum base would be the establishment of a laboratory colony, which could also provide the larvae for the short-exposure testing. However, even with short-exposure tests, a method of determining the instar of live larvae must be available. If partial or complete life-cycle exposures are done, a large data base must be available. During an exposure, subtle changes in developmental events may occur and could be missed if the researcher does not know the sequence, timing, and extent of normal events.

The 1975 edition of *Standard Methods for the Examination of Water and Wastewater* [2] contains tentative bioassay procedures for aquatic insects, including chironomids. A list of chironomid species, including *Chironomus plumosus, Chironomus attenuates, Chironomus tentans, Chironomus californicus, Glyptochironomus labiferus, Goeldichironomus holoprasinus, Tanypus grodhausi,* and *Tanytarsus* (*Paratanytarsus*) *dissimilis,* was provided as a guide to the most commonly tested species. At the U.S. Environmental Protection Agency (EPA) Environmental Research Laboratory in Duluth, Minn., a series of experiments designed to provide baseline biological data for short and life-cycle exposures of *T.* (*P.*) *dissimilis* were recently completed. A quick review of the results of these experiments is provided as an example of the type of information that is needed to develop test protocols.

Life Cycle of *Tanytarsus* (*Paratanytarsus*) *dissimilis*

The general life cycle of chironomids contains these stages: the eggs, four larval instars, pupa, and adult. The egg mass of *T. dissimilis* is cylindrical, up to 5 mm long, with a single row of eggs usually placed diagonally through the center. The mass has a sticky covering, which is quickly covered with debris from the rearing tanks. The eggs are deposited in two

separate masses, usually 1 day apart. An eggs-per-mass distribution of 486 separate masses is shown in Fig. 1. The number of eggs per mass was determined, and the number of egg masses occurring in 10-egg intervals (1 through 10, 11 through 20, etc.) was determined. The first egg mass is the largest and contains from 120 to 190 eggs. The second egg mass is usually deposited the second day after adult emergence and contains 30 to 50 eggs. Only a few masses contain fewer than 30 or more than 200 eggs. Since the eggs are in a single row, they can be separated into pieces with as few as 10 eggs with forceps and a binocular microscope. These small groups can be used to determine hatch success at the end of an exposure or to start a test. The eggs complete development in about 69 h at 18°C. The whole mass has usually completed hatching in about 4 h, with a hatch success of greater than 99 percent.

The just-hatched larva is planktonic for up to a day before settling to a surface and building a tube. The tube is built on a surface, and the insect does not require a burrowing substrate. The tube is constructed of silk, into which algae and other debris are woven. In the laboratory, fecal pellets are often substituted for algae, since this material is not always readily available. As the larva grows, the tube is elongated, with new material added to the end nearest the food source. This laboratory behavior is the same as that reported by Cavanaugh and Tilden [*3*] for the same species. Free-swimming larvae can be seen if the colony is overcrowded or the food supply is low.

The effect of temperature on the growth of the larvae is shown in Fig. 2. In this experiment, the eggs were hatched and the larvae reared at 15, 18, 22 or 28°C to the adult stage. The larvae were measured alive during the exposure. The pupal and adult lengths are also shown in Fig. 2. At 15 or 18°C, the larval development or the time from egg hatch to pupation is

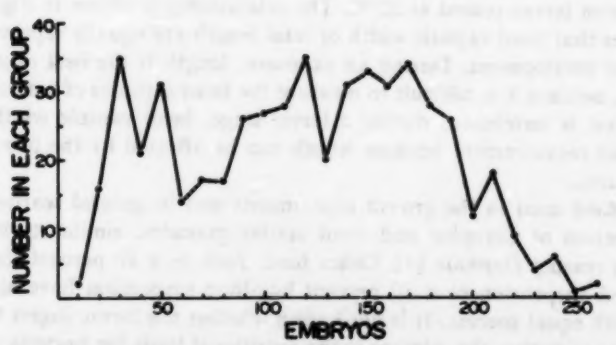


FIG. 1—*Distribution of the number of eggs per mass for 486 egg masses. Each point represents the number of egg masses in each 10-egg interval.*

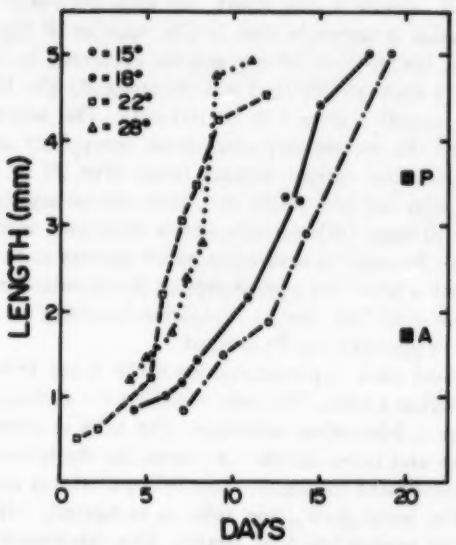


FIG. 2—*Relationship between temperature and larval length. The length of the pupa* (P) *and adult* (A) *are indicated by the solid squares.*

about 18 days. At 22 or 28°C, the larval development is completed in 12 to 14 days. The first instar larva is about 0.6 mm long at hatching. The maximum length of the fourth instar larva is about 5 mm. The pupa is approximately 3.7 mm, and the adult is about 1.7 mm long. Based on this experiment, 22°C seems to be an appropriate temperature for testing.

In another experiment, the head capsule width and length were measured from larvae reared at 22°C. The relationship is shown in Fig. 3 and indicates that head capsule width or total length are equally representative of larval development. During an exposure, length is the best estimate of growth, because it is difficult to measure the head capsules of active larvae. If the test is terminated during a larval stage, head capsule width is the preferred measurement because length can be affected by the termination procedures.

The food used in the growth experiments and in general rearing was a combination of Cerophyl and trout starter granules, similar to the food used in rearing *Daphnia* [*4*]. Other food, such as a 10 percent flake-type fish-food suspension or a 10 percent hog-liver suspension have also been used with equal success. It is not known whether the larvae ingest the food directly or whether the mixture is the nutritional basis for bacteria or algae which are then consumed by the larvae.

A spindle-shaped pupation tube is produced by the fourth instar larva

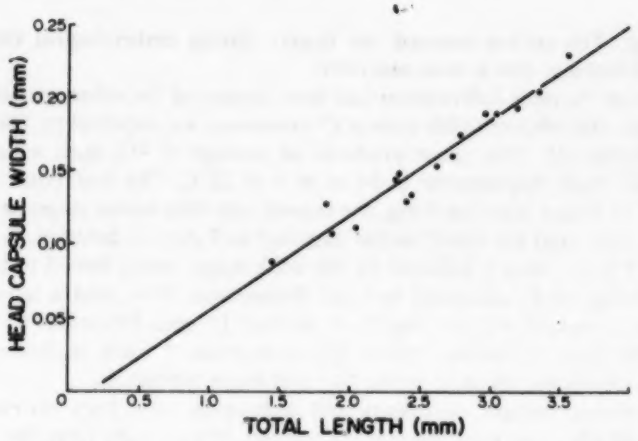


FIG. 3—*Relationship between length and head capsule width for* Tanytarsus (Paratanytarsus) dissimilis.

and is shorter than the larval case. The pupal stage lasts about 48 h at 18°C. At the end of the pupal stage, the pupa wriggles free from the pupal tube and swims to the surface, where adult emergence quickly occurs. In the rearing colony, adult emergence usually occurs over several days, with the major emergence usually occurring during one day.

Most chironomids reproduce sexually, but parthenogenesis, or egg development without the intervention of sperm, has been reported in the subfamily Tanytarsini [*5*]. To determine the extent of parthenogenicity in *T. dissimilis*, pupae were taken from the rearing colony and individually isolated in stoppered glass vials. Of the 82 adults produced from the isolated pupae, 73 (or 90 percent) produced eggs that hatched. The actual incidence may be higher, since some of the adults died before depositing eggs.

The data from these experiments and the experience obtained from routine rearing allowed the selection of appropriate measurements from determining toxicant effects. These measurements have been incorporated into short-exposure, partial life-cycle, and life-cycle toxicity tests. For example, the data show that the first egg mass varies in number from 120 to 190 eggs. This indicates that the number of eggs per mass is perhaps too variable to allow the separation of effect from noneffect concentrations. But, hatching could be used because over 90 percent of the eggs hatch under control conditions, so small changes could be detected. Another example can be seen in the temperature-growth data. Larvae at 20 to 22°C are in the late second or third instar by the tenth day. Based on this data, a partial life-cycle (10-day) test was developed, which was used to determine what concentrations of copper, cadmium, lead, and zinc affected growth or

survival. The system exposed the insects during embryological development, hatching, and at least one molt.

Similar life-cycle information has been developed for other species. For example, the laboratory life cycle of *C. attenuates* was reported by Thornton and Wilhm [*6*]. This insect produces an average of 412 eggs, which will complete their development in 24 to 36 h at 22°C. The first instar larvae molt 2 to 4 days after hatching, the second and third instar stages last 4 to 7 days each, and the fourth instar stage is 5 to 7 days in duration. A pupal stage of 3 to 5 days is followed by the adult stage, which lives 3 to 5 days. The biology of *C. plumosus* in Lake Winnebago, Wis., and a laboratory rearing technique was reported by Hilsenhoff [*7*] and Hilsenhoff and Narf [*8*]. The Lake Winnebago report [*7*] summarizes 7 years of observations and describes the life cycle in the lake and in the laboratory.

Laboratory culture equipment and techniques have been reviewed by Credland [*9*]. The usual equipment consists of a container for the larvae and substrate (if needed) and an enclosed space for the adults. The containers can be plastic refrigerator storage boxes [*10*], coated galvanized metal trays [*6*], or glass aquaria [*11*]. A preferred substrate is sand [*10,12*], although Derr and Zabik [*13*] used a mixture of paper hand towels and chicken feed, which provided both a substrate and food for the larvae. Other foods, such as algae, enriched dog food, powdered cereal, leaves, and flake fish food, have been used successfully [*9,11,14*].[3]

Toxicity Tests

Toxicity tests using chironomids can be placed into three categories—short-exposure, partial life-cycle, and life-cycle tests. This section describes experiments from each category. The details of the experiments are shown in Tables 1 and 2.

Short-Exposure Tests

Short-exposure tests expose only one life-cycle stage. They have an advantage in requiring a minimal biological data base and in providing rapid data production. An example of a short-exposure test is the method developed by Mulla and Khasawinah [*12*]. They exposed fourth instar larvae in waxed paper cups containing a white quartz sand substrate and tap water. Within 1 h after the addition of the larvae, a pesticide was added to the water, and mortality was assessed after 24 h. The results were expressed as 24-h LC_{50} or LC_{90} values. This procedure, which is similar to methods recommended by the World Health Organization [*22*] for toxicity

[3] Biever, K. D., *Annals of the Entomological Society of America*, Vol. 64, 1971, pp. 1166-1169.

TABLE 1—*Outline of chironomid toxicity test procedures: organism, exposure characteristics, and toxicant.*[a]

	Organism			Exposure		Toxicant		
Type of Test	Name	Stage	Food	Length, Days	Effect Measured	Analysis	Mode of Administration	Source, Ref
Short exposure	*Goeldichironomus holoprasinus*	4th instar	none	1 or 2	mortality	N.I.	acetone in tap water	[12]
	Tanypus grodhausi	4th instar	none	1 or 2	mortality	N.I.	acetone in tap water	[12]
	Chironomus sp. 51	4th instar	none	1 or 2	mortality	N.I.	acetone in tap water	[12]
Short exposure	*C. plumosus*	4th instar	none	5 or 7	mortality	N.I.	5 percent technical grade in granules	[15]
Partial life cycle	*C. riparius*	egg	N.A.	3	hatch	N.I.	aerated tap water	[16]
Partial life cycle	*C. plumosus*	larva	dog biscuits and diatoms	21	accumulation	yes	pond water	[17]
	Glyptotendipes paripes	larva	algae	21	accumulation	yes	pond water	[17]
Partial life cycle	*Tanytarsus dissimilis*	egg to 3rd instar	fish food, cereal leaf	10	survival–growth	yes	water	[11]
Partial life cycle	*T. dissimilis*	larval to adult	algae	up to 35	survival	yes	N.A.	[18]
Partial life cycle	*C. attenuatus*	egg to larvae	dog food	21	oxygen consumption	yes	deionized water	[19]
Partial life cycle	*C. tentans*	larva to adult	none added	14+	adult emergence	yes	in sediment	[20]
Life cycle	*T. dissimilis*	egg to adult	algae and cereal leaf	about 18	survival–growth	yes	N.A.	[14]
Life cycle	*C. tentans*	egg to adult	paper hand towel and chicken feed	28 to 35	adult emergence	N.I.	in ethanol	[13]
Life cycle	*C. tentans*	egg to adult	paper hand towel and chicken feed	25 to 30	accumulation	yes	in ethanol	[21]
Life cycle	*C. attenuatus*	egg to adult	dog food	until 4th instar	caloric content lipid, protein survival	yes	deionized water	[6]

[a] N.A. = not applicable; N.I. = not indicated.

TABLE 2—*Outline of chironomid toxicity tests procedures: physical and chemical characteristics.*[a]

Type of Test	Temperature, deg. C	Photo-period, h	Light	Routine Chemistry Type	Tank Size	Tank Material	Flow	Volume	Aera-tion	Sub-strate	Source, Ref
Short exposure	26 ± 1	N.I.	N.I.	N.I.	120 ml (4 oz)	waxed paper	no	100 ml	no	yes	[12]
Short exposure	20	N.I.	N.I.	N.I.	57 litres (15 gal)	glass-slate	no	40 litres	yes	yes	[15]
Partial life cycle	21	N.I.	N.I.	pH, DO	7 ml (2 dram)	glass	no	N.I.	no	N.A.	[16]
Partial life cycle	23	N.I.	N.I.	N.I.	N.I.	polyethylene	15 ml/h	N.I.	yes	yes	[17]
Partial life cycle	22 ± 1	16	fluorescent at 20 footcandles (215 lx)	pH, DO, Hd, Alk	400-ml beaker	glass	no	200 ml	no	no	[11]
Partial life cycle	18.5 ± 1	16	fluorescent at 162 footcandles (1744 lx)	DO, Hd, Alk	30 litres (8 gal)	glass	no	N.I.	no	no	[18]
Partial life cycle	21 to 25	N.I.	N.I.	N.I.	10 litres	plastic	yes	5 litres	yes	yes	[19]
Partial life cycle	22 ± 1	N.I.	N.I.	Alk, Hd, pH	4 litres	N.I.	no	2 litres	yes	yes	[20]
Life cycle	N.A. (temperature test)	16	fluorescent at 60 footcandles (646 lx)	Alk, pH, Hd	6-cm dish	aluminum	no	22 ml	no	no	[14]
Life cycle	N.I.	N.I.	N.I.	N.I.	45 or 4 litres	glass	no	N.I.	yes	yes	[13]
Life cycle	21	12	fluorescent	conductivity, DO, Alk Hd, pH	3 litres	glass	110 ml/min	3 litres	no	yes	[21]
Life cycle	24 ± 0.5	12	fluorescent at 290 footcandles (3122 lx/cm^2)	N.I.	quart Mason jars (1 litre)	glass	1 replacement in 0.66 days	1 litre	yes	N.I.	[6]

[a] N.A. = not applicable; N.I. = not indicated.

evaluations of mosquito larvicides, has been extensively used [*23–25*] to determine the efficacy of insecticides in controlling nuisance emergences.

A natural mud substrate and fourth instar *C. plumosus* were used by Hilsenhoff [*15*] to determine the toxic effect concentrations of 16 pesticides. The tests were conducted in slate-bottom aquaria that contained 6 cm of mud. Fifty larvae were added and allowed 1 h to burrow into the mud before the formulated insecticide was distributed over the water surface. After 5 or 7 days, the mud was strained, and the number of living larvae was recorded, and the percent mortality was calculated.

Partial Life-Cycle Tests

Partial life-cycle tests expose the insect through at least one developmental event such as hatching or molting. These tests combine the shorter exposure advantages of the acute test with the toxicological advantage of exposing the insect through hatching, molting, pupation, or emergence.

Partial life-cycle exposures usually start with eggs or larvae and may continue through to adult emergence. Because of this variability, a general description of these types of tests is not possible. The eggs of *C. riparius* were exposed to calcium sulfide during embryological development and hatching by Wegner and Hamilton [*16*], and the percent hatch was calculated. "Mature" or second instar larvae were used by Wentsel et al [*20, 26*] to determine the effect of heavy-metal-contaminated sediment on larval survival or adult emergence. In these tests, laboratory-reared larvae were simply added to the contaminated sediment, and the larval survival or the adult emergence was determined and compared with data from a control sediment. Exposures that included several molts have been reported by Bell [*18*], Harvey [*17*], Cole and Wilhm [*19*], and Anderson et al [*11*]. Bell exposed *T. dissimilis* to five low-pH concentrations. The exposures started with larvae and continued through to the adult stage. Cole and Wilhm [*19*] exposed *C. attenuatus* from the egg through to the fourth instar to phenol and determined its effect on fourth instar larva oxygen consumption. Another report [*11*] reported the effect of four heavy metals on *T. dissimilis*. The test was started with eggs and continued to the second or third instar larva stage with both growth and survival used as determinants of effect. A flow-through exposure of radionuclides to *Glyptotendipes paripes* and *C. plumosus* [*17*] started with larvae and continued to adult emergence.

Life-Cycle Tests

Life-cycle tests expose the insect through all its life stages. The usual life-cycle exposure approach is to start with eggs and continually expose the insect through the life cycle, including the stages of adult emergence and subsequent egg deposition and hatching. Two reports by Derr and Zabik

[*13,21*] describe a procedure for exposing *C. tentans* from egg to adult emergence. These studies placed eggs in a highly organic sediment and either sampled during larval development or collected the adults after 25 to 30 days. Temperature effects on the life cycle of *T. dissimilis* were reported by Nebeker [*14*]. The larvae were measured during the test, and developmental rates for the embryo, larvae, pupae, and adults at six temperatures were obtained. Effects of pH, phenol, and sodium chloride on *C. attenuatus* were reported by Thornton and Wilhm [*6*]. The exposed populations were extensively monitored for survival, lipid content, protein, and caloric content during the exposure.

Discussion

Chironomids occupy a unique position in the considerations of a regulatory aquatic biologist, because they are both an essential component of freshwater ecosystems and, under certain conditions, a pest to humans. The effect of this dual role of chironomids is reflected in this survey of toxicity reports. Each researcher has selected a species and developed a test procedure that provides answers to his specific objectives. For example, the need for controlling nuisance emergences of chironomids was instrumental in developing the procedures used by Hilsenhoff [*15*] and Mulla and Khasawinah [*12*]. These procedures could also be used by the regulatory biologist to determine the comparative toxicity of, for example, a series of industrial chemicals. But the short-exposure techniques might not be adequate if the objective is to determine a specific concentration that would not affect any developmental stage. In this situation, a complete life-cycle exposure would be necessary. These tests require a much larger biological data base than that required for the short-exposure tests.

The lack of biological data can limit the use of partial or complete life-cycle tests. At this time, the species most often used are *C. plumosus, C. tentans, C. attenuatus, C. riparius,* and *T. (P.) dissimilis.* The life-cycle data base is larger for these chironomids than for others, but this should not exclude the use of other species. New species and new life-cycle data are always needed.

In summary, chironomids have the useful characteristics of easily identifiable life stages, a short laboratory life cycle, and a reasonably large biological data base. What is needed is the promotion of these animals as toxicity test organisms by using them in tests and, ultimately, in the establishment of standard test procedures.

References

[*1*] Oliver, D. R., *Annual Review of Entomology,* Vol. 16, 1971, pp. 211-230.
[*2*] *Standard Methods for the Examination of Water and Wastewater,* 14th ed., American

Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C., 1976, pp. 827–834.
[3] Cavanaugh, W. J. and Tilden, J. E., *Ecology*, Vol. 11, 1930, pp. 281–287.
[4] Biesinger, K. E. and Christensen, G. M., *Journal of the Fisheries Research Board of Canada*, Vol. 29, 1972, pp. 1691–1700.
[5] Edwards, D. H. D., *Proceedings of the Royal Entomological Society of London*, Vol. 38, 1963, pp. 165–170.
[6] Thornton, K. and Wilhm, J., *Hydrobiologia*, Vol. 45, 1974, pp. 261–280.
[7] Hilsenhoff, W. L., *Annals of the Entomological Society of America*, Vol. 60, 1967, pp. 1183–1194.
[8] Hilsenhoff, W. L. and Narf, R. P., *Mosquito News*, Vol. 27, 1967, pp. 363–366.
[9] Credland, P. F., *Freshwater Biology*, Vol. 3, 1973, pp. 45–51.
[10] Biever, K. D., *Annals of the Entomological Society of America*, Vol. 58, 1965, pp. 135–136.
[11] Anderson, R. L., Walbridge, C. T., and Fiandt, J., *Archives of Environmental Contamination and Toxicology*, Vol. 9, 1980, pp. 329–335.
[12] Mulla, M. S. and Khasawinah, A. M., *Journal of Economic Entomology*, Vol. 62, 1969, pp. 37–41.
[13] Derr, S. K. and Zabik, M. J., *Bulletin of Environmental Contamination and Toxicology*, Vol. 7, 1972, pp. 366–368.
[14] Nebeker, A. V., *Journal of the Kansas Entomological Society*, Vol. 46, 1973, pp. 160–165.
[15] Hilsenhoff, W. L., *Journal of Economic Entomology*, Vol. 52, 1959, pp. 331–332.
[16] Wegner, G. S. and Hamilton, R. W., *Environmental Entomology*, Vol. 5, 1976, pp. 256–258.
[17] Harvey, R. S., *Health Physics*, Vol. 20, 1971, pp. 613–616.
[18] Bell, H., *Canadian Entomologist*, Vol. 102, 1970, pp. 636–639.
[19] Cole, S. L. and Wilhm, J., *Water Research*, Vol. 7, 1973, pp. 1691–1700.
[20] Wentsel, R., McIntosh, A., and McCafferty, W. P., *Hydrobiologia*, Vol. 57, 1978, pp. 195–196.
[21] Derr, S. K. and Zabik, M. J., *Transactions of the American Fisheries Society*, Vol. 101, 1972, pp. 323–329.
[22] "Insect Resistance and Vector Control," 8th Report of the Expert Committee, *Technical Report Series*, No. 153, World Health Organization, Geneva, Switzerland, 1957.
[23] Mulla, M. S., Norland, R. L., Ikeshoji, T., and Kramer, W., *Journal of Economic Entomology*, Vol. 67, 1974, pp. 165–170.
[24] Ali, A. and Mulla, M. S., *Journal of Economic Entomology*, Vol. 69, 1976, pp. 509–513.
[25] Ali, A. and Mulla, M. S., *Journal of Economic Entomology*, Vol. 71, 1978, pp. 778–782.
[26] Wentsel, R., McIntosh, A., and Atchison, G., *Hydrobiologia*, Vol. 56, 1977, pp. 153–156.